

Amendments to the Specification:

Please replace the paragraph beginning at page 7, line 18, with the following red-lined paragraph:

Figure 2 is a graphical representation of the characterization of the molecular weight of HA synthesised and differential expression of the hyaluronidase genes by parental, mock and HAS2 antisense transfected MDA-MB 231. A: Cells were seeded at 7.5×10^5 cells in 75cm² culture flasks and grown for 24h in complete medium supplemented with 5μCi of D-[6-³H]-glucosamine hydrochloride. To determine the MW of ³H-HA in the medium, samples were subjected to size exclusion chromatography on a Sephacryl® S-1000 SF eluted in 0.15M NaCl/phosphate pH 7.25 at 13.6ml/h. This figure demonstrates the differences in molecular weight synthesized by parental MDA-MD 231 and their transfected counterparts harbouring antisense mRNA to HAS2. B: Total RNA extracted from parental, mock and ASHAS2 transfected MDA-MD 231 was analysed by RT-PCR to detect the levels of the hyaluronidase genes, notable HYAL-1,2 and 3. PCR products were resolved by agarose gel electrophoresis containing ethidium bromide. Stained gels were then subjected to densitometric analysis to allow comparison of levels for each HYAL gene between parental, mock and ASHAS2 transfected cells. Note, both parental and mock-transfected MDA-MD 231 cells express comparable levels of both HYAL-1 and 2 but do not express HYAL-2. In contrast ASHAS2 transfected cells, HYAL-2 is not expressed whereas HYAL-3 was detected and HYAL-1 was moderately increased in expression.

Please replace the paragraph beginning at page 8, line 26, with the following red-lined paragraph:

Figure 5 is a graphical representation of the effect of antisense inhibition of HAS2 on cell cycle. The transfected and control cells were seeded at 2×10^5 cells/25cm² flask in the presence of 2mM thymidine and grown until 50% confluent. Cells were washed then returned to normal culture medium and harvested, by trypsinisation, at the following time points; 0h, 4h, 8h, 12h, 16h, 20h, 24h, 28h, 32h, and 36h then fixed in 95% w/v ethanol for 2h at 4°C. Cells were pretreated with 100μg/mL RNAase (Sigma) and 50μg/ml propidium iodide (Sigma) for 30minutes at 37°C before determining the cell cycle stage in a FACS-Calibur™ (Trade Mark)

analytical instrument (Becton Dickinson, San Jose, CA). Panel A: population of cells in G₀/G₁; Panel B: in S phase, and PANEL C: in G₂/M phase. Note the delay of 24 hours of entry into S PHASE in the ASHAS2 MDA MB 231 transfectants.

Please replace the paragraph beginning at page 10, line 13, with the following red-lined paragraph:

Figure 9 is a diagrammatic representation demonstrating comparison of the invasive potential of human breast cancer cell lines and HA receptors. The invasive potential of the breast cell lines (*-*) were examined using the Boyden chamber chemoinvasion assay as described in Materials and Methods. The cells that had traversed the matrigel and spread on the lower surface of the filter were expressed as a percentage of the cell count determined for the Hs578T cell line. The data presented represents the mean SD average of triplicate experiments performed on two separate days. Note: percentage variance between triplicate determinations < 2%. Quantitation of HA receptors (A) CD44 and (B) RHAMM was determined by immunoblotting where immunoreactive bands were quantified by densitometry analysis using ProXpressTM [trade mark] Imager and the data analysed using PhoretixTM 1 D software.

Please replace the paragraph beginning at page 124, line 14, with the following red-lined paragraph:

The cDNA for human HAS2 was generated by designing gene specific primers from the published sequence of Watanabe and Yamaguchi (1996; Genbank accession no. U54804) and consisted of the following primers: sense 5'>GAGCTGAACAAGATGCATTGTGAGAGC (SEQ ID NO: 1) and antisense 5'GACATGGTGCTT-GATGTATGATCTTCCAT (SEQ ID NO: 2). Total RNA harvested from exponentially dividing human dermal fibroblasts was used as the template for RT-PCR to generate a 1.7kb cDNA fragment of HAS2, which was cloned directly into pGEM[®]-T vector (Promega Corporation, Madisom, USA). The cDNA for HAS2 was subsequently subcloned into the pCI-Neo expression vector (Promega Corporation, Madisom, USA) and isolated clones containing the insert in the antisense orientation were identified by restriction endonuclease mapping and automated sequencing.

Please replace the paragraph beginning at page 124, line 29, with the following red-lined paragraph:

The ASHAS2-pCl-Neo construct and pCl-neo vector were transfected into human MDA-MB 231 breast cells using Lipofectamine™ plus reagent (Gibco life technologies, USA) according to the manufacture's instructions. Prior to commencing studies transfected cells were selected for at least one month in the presence of 500pg/mL G418 antibiotic.

Please replace the paragraph beginning at page 125, line 20, with the following red-lined paragraph:

Real time PCR was used to quantitate the relative mRNA levels of HAS1, HAS2, and HAS3 in parental, mock and ASHAS2 transfected cells using gene specific primers and an internal oligonucleotide probe. In brief, total RNA was purified from experimental cells using Rneasy® (Qiagen, Melbourne, Australia) which was then used to generate single stranded cDNA by incubating 2µg RNA with 0.5µg/uL random primers and superscript reverse transcriptase. For quantitative real time PCR gene specific primers for each HAS isoform and an internal oligonucleotide probe were used. In brief, the primers consisted of the following : HAS1, sense, 5'CCTGCATCAGCGGTCCTCTA 3' (SEQ ID NO: 6); HAS1 antisense, 5'GCCGGTCA-TCCCCAAAAG 3' (SEQ ID NO: 7); HAS1 probe, 5' AACCTCTTGCAGCAGTTTCTTGAGGCC 3' (SEQ ID NO: 8); HAS2 sense, 5' CAGTCCTGGCTTCGAGCAG 3' (SEQ ID NO: 9); HAS2 antisense, 5' TTGGGAGAAAAGTCTTTGGCT 3' (SEQ ID NO: 10); HAS2 probe, 5' CCATTGAACCAG-AGACTTGAAACAGCCC 3' (SEQ ID NO: 11); HAS3 sense, 5' TTGCACTGTGGTCGTCAACTT 3' (SEQ ID NO: 12); HAS3 antisense, 5' GTCGAGGTCAAACGTTGTGAG 3' (SEQ ID NO: 13); HAS3 probe, 5' TCAAATCAAAAACAGGCAGGTACAGGTAGTGG 3' (SEQ ID NO: 14); GAPDH sense, 5'AAGGTGAAGGTCGGAGTCAAC 3' (SEQ ID NO: 15); GAPDH antisense, 5' GAGTTAAAA-GCAGCCCTGGTG 3' (SEQ ID NO: 16); GAPDH probe, 5' TTTGGTCGTATTGGGCGCCTGG3' (SEQ ID NO: 17). For HAS internal probes the reporter

dye 6-carboxylfluorescein (6-FAM[™]) and quencher 6-carboxytetramethyl rhodamine (TAMRA[™]) were labelled at the 5' and 3' respectively. For GAPDH internal probes the reporter 6-FAM[™] was substituted with VIC[™] (Applied Biosystems California, USA). The PCR reaction was performed in a final volume of 30pL and consisted of 1x Taqman reaction mix, 6μM of HAS forward and reverse primer, 1.5pM of probe, 1pM of each GAPDH primer and 500nM of GAPDH probe. PCR amplification was by denaturation for 10 minutes at 95°C followed by annealing for 2 minutes at 50°C followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Thermocycling and fluorescence measurement were performed in a ABI Prism 7700[®] sequence detection system (Applied Biosystems, California, USA). To allow comparison between samples the relative hyaluronan synthase signals were normalised with internal GAPDH control measurements.

Please replace the paragraph beginning at page 127, line 18, with the following red-lined paragraph:

To allow immunodetection and comparison between parental, mock and ASHAS2-pCNeo transfected MDA-MB-231 cells specific antibodies to HAS2 and Hyal2 were kindly gifted from Dr Paraskevi Heldin and Dr Robert Stern respectively. Anti-human CD44 Clone DF1485 was purchased from DAKO (Denmark) and used according to manufacturers instructions. Cells were seeded into 8-well chamber slides at a density of 2×10^4 cells/well and grown for a further 24 hours at 37°C. The cells were fixed in Histochoice[®] for 15 minutes before blocking heterophile proteins by incubation in PBS containing 10% FCS. The primary antibodies were diluted to (cite concentration NOT dilution) in antibody diluent (PBS containing 1% human serum and 1% FCS) then incubated on slides for 60min at room temperature. Endogenous peroxidase activity was blocked by immersion of slides in 0.3% H₂O₂ in methanol for 20min prior to incubation with a peroxidase-conjugated rabbit anti-sheep secondary antiserum for 60min at RT. The immunocomplexes were visualised by applying 3,3'-Diaminobenzidine substrate (Sigma Fast DAB) for 5-10 minutes, then counterstained with haematoxylin, dehydrated and mounted.

Please replace the paragraph beginning at page 128, line 18, with the following red-lined paragraph:

The Boyden chamber chemoinvasion assay was performed as described previously (Thompson et al., 1992). Matrigel® (50µg) was dried onto polycarbonate filters (12µm pore, PVP free, Nucleopore, Pleasanton, CA) and then reconstituted at 37°C. Normal growth media (L-15 medium) containing 0.1% bovine serum albumin (Miles Biochemicals, Kankakee, IL) was used as the chemoattractant. Cells were harvested in the logarithmic growth phase by trypsinisation, washed twice with serum-free L-15 medium containing 0.1% bovine serum albumin then seeded at 300,000 cells/1ml chamber and 70,000 cells/0.2ml chamber. Each experiment was performed in triplicate. Chambers were incubated in a humidified incubator at 37°C for 6 hours. To determine the population of cells which had traversed the ~~matrigel~~Matrigel®, the filters were stained with Diff-Quik® (American Scientific Products, McGaw PK. , IL) then counted.

Please replace the paragraph beginning at page 130, line 7, with the following red-lined paragraph:

Parental, mock and ASHAS2 transfected cells were harvested in the logarithmic growth phase by scraping. Cells were resuspended to a final density of 2×10^6 cells in L-15 medium supplemented with 0.1% glucose +/-Matrigel® (v/vWHAT IS THE PERCENTAGE?) then immediately injected into the mammary fat pad of 5 weeks old female CBA nude mice (n=11 does each treatment group consist of 11). Tumor growth was recorded twice weekly by measuring three perpendicular diameters (d1, d2, d3). Tumor volume was then calculated using the formula: $(1/6)\pi (d1d2d3)$. On the day 84 mice were humanely killed and liver, kidneys, brain and lungs removed at autopsy and stored at -20°C. For histological examination half of the primary tumor was fixed in 4% formaldehyde and embedded in paraffin, then 5µm sections from this tissue was examined by H&E staining. The remaining portion of the tumor was frozen at -20°C until further analysis.

Please replace the paragraph beginning at page 130, line 23, with the following red-lined paragraph:

Quantitative Alu PCR was used to detect metastasis of MDA-MD 231 from the primary tumor to other organs collected at autopsy. In brief, DNA was extracted by grinding samples under liquid nitrogen and resuspending in a DNA lysis buffer. DNA was then purified using standard phenol-chloroform methodology followed by ethanol precipitation and reconstitution in TE buffer. The purified DNA was adjusted to a final concentration of 10ng/pL in TE buffer pH7.2, aliquoted and stored at -20°C until analysis. To remove exogenous human DNA contamination the reaction mix was treated with 17U/ml nuclease S7 (Roche) in the presence of 1mM CaCl₂ at 37°C for 24 hours prior to PCR. Quantitative Alu PCR was then performed on purified genomic DNA samples (10ng) in a GeneAmp 5700® Sequence Detection System (Applied Biosystems, Australia). In brief, each sample was tested in duplicate in a final reaction volume of 25µL consisting of 0.625U Taq DNA polymerase (Roche; Mannheim, Germany), 10mM Tris-HCl pH 8.3, 1.5mM MgCl₂, 50mM KCl, 200µM dNTPs, 8% DMSO, 1 pg/ml 6-carboxy-X-rhodamine (Molecular Probes; Eugene, Oregon USA), 1 in 40000 dilution of SYBR Green I (Molecular Probes) and 100nM of each Alu primer. Following an initial denaturation incubation at 95°C for 2 minutes, amplification occurred over 40 cycles, which consisted of denaturation at 95°C for 5 seconds, annealing at 65°C for 60seconds, and extension at 75°C for 15 seconds during which the intensity of fluorescence was measured. A dissociation curve was then generated from 60°C to 95°C. On each 96-well reaction plate, a standard curve was prepared by serially diluting human DNA into mouse DNA which permitted the quantification of the tissue burden of human tumor cells in the mouse organs removed at autopsy.

Please replace the paragraph beginning at page 133, line 15, with the following red-lined paragraph:

The molecular mass of HA synthesised by parental, mock and antisense transfected cells was determined by Sephacryl® S-1000 size exclusion chromatography. The parental cell line synthesised three distinct molecular weights of HA estimated to be 3000 kDa, 40,000 and 100,000 Da respectively which reflects the products of the HAS isoforms expressed in the

parental cell line, notably HAS2 and 3. Antisense HAS2 transfectants synthesised HA which was eluted in the void volume that corresponds to a molecular weight $>1.67 \times 10^7$.

Please replace the paragraph beginning at page 137, line 13, with the following red-lined paragraph:

Real time and comparative reverse transcriptase PCR were used respectively to quantitate the relative mRNA levels of the HA synthases (HAS1-3) in the ten human breast cancer cell lines by using gene specific primers and an internal oligonucleotide probe (Table 2). RNA was extracted from triplicate cultures of cells grown to both exponential and plateau phase using RNeasy® Mini Kits (QIAGEN, Basel, Switzerland). In brief, total RNA was purified from exponentially growing cells using TRI-reagent® (Sigma) which was used to generate single stranded cDNA by incubating 2µg RNA with 0.5µg/µl random primers and ~~superscript~~ Superscript™ reverse transcriptase (Invitrogen, Carlsbad, CA, USA). For quantitative real time PCR gene specific primers for each HAS isoform and an internal oligonucleotide probe were used. For HAS internal probes the reporter dye 6-carboxylfluorescein (6-FAM™) and quencher 6-carboxytetramethyl rhodamine (TAMRA™) was labelled at the 5' and 3' respectively. For GAPDH internal probes the reporter 6-FAM™ was substituted with VIC™ (Applied Biosystems, Foster City, CA, USA). The PCR reaction was performed in a final volume of 30µl and consisted of 1x Taqman reaction mix, 6µM of HAS forward and reverse primer, 1.5µM of probe, 1µM of each GAPDH primer and 500nM of GAPDH probe. PCR amplification was performed by denaturation for 10 min at 95°C followed by annealing for 2 min at 50°C followed by 40 cycles of 15 seconds at 95°C and 1 min at 60°C. Thermocycling and fluorescence measurement were performed in an ABI Prism 7700® sequence detection system (Applied Biosystems). Relative quantitation was performed by normalizing threshold cycle (Ct) values of each sample gene with Ct values of the GAPDH. ΔC_t corresponds to the difference between the Ct of the HAS genes of interest and the Ct of the GAPDH. Data are presented as fold-change difference relative to parental (arbitrarily set to 100) calculated according to the formula describing relative PCR quantitation $2^{-(\Delta C_t \text{HAS} - \Delta C_t \text{GAPDH})}$.

Please replace the paragraph beginning at page 138, line 8, with the following red-lined paragraph:

To determine the hyaluronidase gene expression for HYAL-1, 2 and 3, RT-PCR was performed on total RNA extracted from cells in both the exponential and growth arrested phases. The gene specific primer sets were designed from sequences retrieved from GenBank® (refer Table 2). Amplified sequences were visualised by agarose gel electrophoresis containing ethidium bromide and their identity confirmed by automated DNA sequencing. To quantitate the relative abundance of each PCR product, ethidium bromide stained agarose gels containing amplified fragments were subjected to densitometric analysis using ProXpress™ Imager (Perkin Elmer, Boston, MA, USA) and the data analysed using Phoretix™ 1 D software (Phoretic International, Newcastle, UK).

Please replace the paragraph beginning at page 125, line 20, with the following red-lined paragraph:

Cells were seeded at 7.5×10^5 cells/75cm² culture flask and were grown for 24h in growth media containing 400µg/ml DS and 250pCi D-[6-³H] glucosamine hydrochloride (Perkin Elmer, Boston, MA, USA). At the conclusion of the 24h incubation period, the media was removed and exhaustively dialysed (Mr exclusion of 6 kDa) against 10mM Tris-HCl/0.15M sodium chloride/0.02% sodium azide pH 7.4 at 4°C. The dialysate and dialysis fluid were chromatographically analysed for the identification of [³H] HA and its degradation products. [³H] HA of >5kD was subjected to size exclusion chromatography in a Sephacryl® S-1000 gel eluted in 0.15M NaCl/phosphate pH7.25 which contained 19mM NaH₂PO₄, 38mM Na₂HPO₄ and 94mM NaCl at 13.6ml/h. The dialysis fluid (molecules <5kD) was subjected to size exclusion chromatography in a Superose® 12 gel eluted in the above-mentioned buffer at an elution rate of 20ml/h. Molecular weight estimations were calculated using calibration data for HA in Sephacryl® S-1000 and Superose® 12 data generated from commercially purchased HA fractions of high monodispersity ranging from 10k to 5000 kDa (CPN, Czech Republic and Pharmacia). To determine the percentage incorporation D-[6-³H] glucosamine hydrochloride

into Ha macromolecules, the non-dialysable (molecules >5 kDa) dpm was subjected to digestion by 10 TRU of Streptomyces hyaluronidase at pH 6, 37°C for 24h. Digested material was subjected to chromatography in both Sephacryl® S-1000 and Superose® 12 where profiles were compared to equivalent undigested sample. Any [³H] material not digested by hyaluronidase was excluded from the chromatography profiles. For the calculation of column recoveries, counts in each fraction were taken as significant when >3 S.D. above the mean background dpm, with the background determined taking an equal number of sample points before and after V_o and V_t, where the average number taken was 20.

Please replace the paragraph beginning at page 141, line 21, with the following red-lined paragraph:

Cell extracts were obtained by hypotonic lysis of exponentially growing cells in 10mM HEPES pH 7.2 followed by disruption in a Dounce homogeniser using 20 strokes every 15 minutes. Cell lysis was confirmed by Giemsa stain of cell lysate and examination by light microscopy. Cell lysate preparations were denatured at 65°C for 5 min and loaded (15-30 pg of protein per lane) onto a 10% polyacrylamide gel. Electrophoresis was performed on a Bio-Rad minigel apparatus. Proteins were transferred to nitrocellulose membranes and blocked for 1 h with Tris-buffered saline containing 5% nonfat dry milk and 0.1% Tween-20. Membranes were then washed and probed with the appropriate antibody diluted in Tris-buffered saline containing 5% bovine serum albumin (for polyclonal antibodies) or 5% nonfat dry milk (for monoclonal antibodies). The antibodies used for detection were 50µg of CD44s monoclonal antibody (Hybridoma Bank, USA) or 25pg RHAMM (kindly donated by R. Savani, University of Pennsylvania School of Medicine, USA). The secondary antibodies used were anti-rabbit IgG (New England Bio-labs) and rabbit anti-rat IgG (Bio-Rad), which were conjugated with horseradish peroxidase. Immunoreactive bands were detected by enhanced chemiluminescence, and the sizes of proteins were estimated using prestained molecular weight standards. Immunoreactive bands were quantified by densitometry. analysis using ProXpress™ Imager (Perkin Elmer, Boston, MA, USA) and the data analysed using Phoretix™ 1D software (Phoretic International, Newcastle, UK).

Please replace the paragraph beginning at page 150, line 16, with the following red-lined paragraph:

The cDNA open reading frame for human HAS2 was generated by designing gene specific primers from the published sequence of Watanabe and Yamaguchi and consisted of the following primers: sense, 5'-GAGCTGAACAAGATGCATTGTGAGAGC-3' (SEQ ID NO: 45) and antisense, 5'-GACATGGTGCTTGATGTATGATCTTCCAT-3' (SEQ ID NO: 46). Total RNA harvested from exponentially dividing human dermal fibroblasts was used as the template for RT-PCR, generating a 1.7kb cDNA fragment of HAS2, which was cloned directly into pGEM®-T vector (Promega, Madison, USA). The cDNA for HAS2 was subsequently subcloned into the pCl-neo expression vector (Promega) and isolated clones containing the insert in the antisense orientation (ASHAS2 construct) were identified by restriction endonuclease mapping and automated sequencing.

Please replace the paragraph beginning at page 150, line 29, with the following red-lined paragraph:

The ASHAS2-pCI-Neo construct and mock control (pCl-neo vector without insert) were transfected into human MDA-MB 231 breast cancer cells using Lipofectamine™ plus reagent (Gibco Life Technologies, Melbourne, Victoria, Australia) according to the manufacturer's instructions. For at least one month, prior to commencing studies, transfected cells were selected in the presence of 500ug/ml G418 antibiotic (Promega). Stable cell lines were established by harvesting and pooling of antibiotic-resistant colonies. Confirmation of the stable incorporation of the antisense HAS2 construct into the genome was performed using PCR on purified genomic DNA. In brief, a gene specific primer for pCl-neo: 5'-GCACAGATGCGTAAGGAG-3' (SEQ ID NO: 47) was used in combination with two specific HAS2 primers of the following sequence: GSP2 sense 5'-GCTGTGTACATGACCTCGCGCTTGCCGCC-3' (SEQ ID NO: 48) and GSP4 sense, 5'-GGCGGGAAGTAAACTCGAC-3' (SEQ ID NO: 49). When used in the following combination; pCl-neo/GSP2 and pCl-neo/GSP4, expected size products of 1443bp and 2223bp

were amplified respectively. The products of PCR were identified by restriction endonuclease mapping and automated sequencing.

Please replace the paragraph beginning at page 151, line 11, with the following red-lined paragraph:

Real time PCR using gene specific primers and an internal oligonucleotide probe was used to quantitate the relative mRNA levels of HAS1, HAS2, and HAS3 in parental, mock and ASHAS2 transfected cells (Table 2). In brief, total RNA was purified from exponentially growing cells using TRI-reagent (Sigma). The total RNA was used to generate single stranded cDNA by incubating mg RNA with 0.5µg/µl random primers and superscript reverse transcriptase (Invitrogen, Carlsbad, CA, USA). For quantitative real time PCR gene specific primers for each HAS isoform and an internal oligonucleotide probe were used. For HAS internal probes the reporter dye 6-carboxylfluorescein (6-FAM[™]) and quencher 6- carboxytetramethyl rhodamine (TAMRA[™]) was labelled at the 5'and 3'respectively. For GAPDH internal probes the reporter 6-FAM[™] was substituted with VIC[™] (Applied Biosystems, Foster City, CA, USA). The PCR reaction was performed in a final volume of 30p1 and consisted of 1x Taqman reaction mix, 6µM of HAS forward and reverse primer, 1.5µM of probe, 1M of each GAPDH primer and 500nM of GAPDH probe. PCR amplification was performed by denaturation for 10 min at 95°C followed by annealing for 2 min at 50°C followed by 40 cycles of 15 seconds at 95°C and 1 min at 60°C. Thermocycling and fluorescence measurement were performed in an ABI Prism 7700® sequence detection system (Applied Biosystems). Relative quantitation was performed by normalizing threshold cycle (Ct) values of each sample gene with Ct values of the GAPDH. ΔCt corresponds to the difference between the Ct of the HAS genes of interest and the Ct of the GAPDH. Data are presented as fold-change difference relative to parental (arbitrarily set to 100) calculated according to the formula describing relative PCR quantitation $2^{-(\Delta C_{tHAS} - \Delta C_{tGAPDH})}$.

Please replace the paragraph beginning at page 152, line 5, with the following red-lined paragraph:

To determine the hyaluronidase gene expression for HYAL1, 2 and 3, RT-PCR was performed on total RNA extracted from cells in both the exponential and growth arrested phases. The gene specific primer sets were designed from sequences retrieved from GenBank® (refer Table 2). Amplified sequences were visualised by agarose gel electrophoresis containing ethidium bromide and their identity confirmed by automated DNA sequencing. To quantitate the relative abundance of each PCR product, ethidium bromide stained agarose gels containing amplified fragments were subjected to densitometric analysis using ProXpress™ Imager (Perkin Elmer, Boston, MA, USA) and the data analysed using Phoretix™ 1D software (Phoretic International, Newcastle, UK).

Please replace the paragraph beginning at page 153, line 19, with the following red-lined paragraph:

Cell migration assay. Invasion assays were performed using modified Boyden chambers with polycarbonate Nucleopore membranes (Corning, Coming, NY, USA). Pre-coated filters (6.5 mm in diameter, 12µm pore-size, Matrigel® 100µg/cm²) were rehydrated with 100µl of Leibovitz L-15 media supplemented with 0.1% w/v BSA (Sigma). Exponentially growing cells were harvested with trypsin/EDTA (Sigma). Before addition to the top chamber of the Boyden apparatus, 3×10^5 cells/ml chamber were washed twice with serum-free growth medium containing 0.1% w/v BSA. Normal growth media containing 10% v/v FCS was used as the chemo attractant. After incubation for 6h at 37°C, non-invaded cells on the upper surface of the filter were wiped with a cotton swab, and migrated cells on the lower surface of the filter were fixed and stained with Diff-Quick® kit. Invasiveness was determined by counting cells in five microscopic fields per well, and the extent of invasion was expressed as an average number of cells per microscopic field. Each experiment was performed in triplicate on two separate days where data is represented as % of migrating cells compared to the parental cell line.